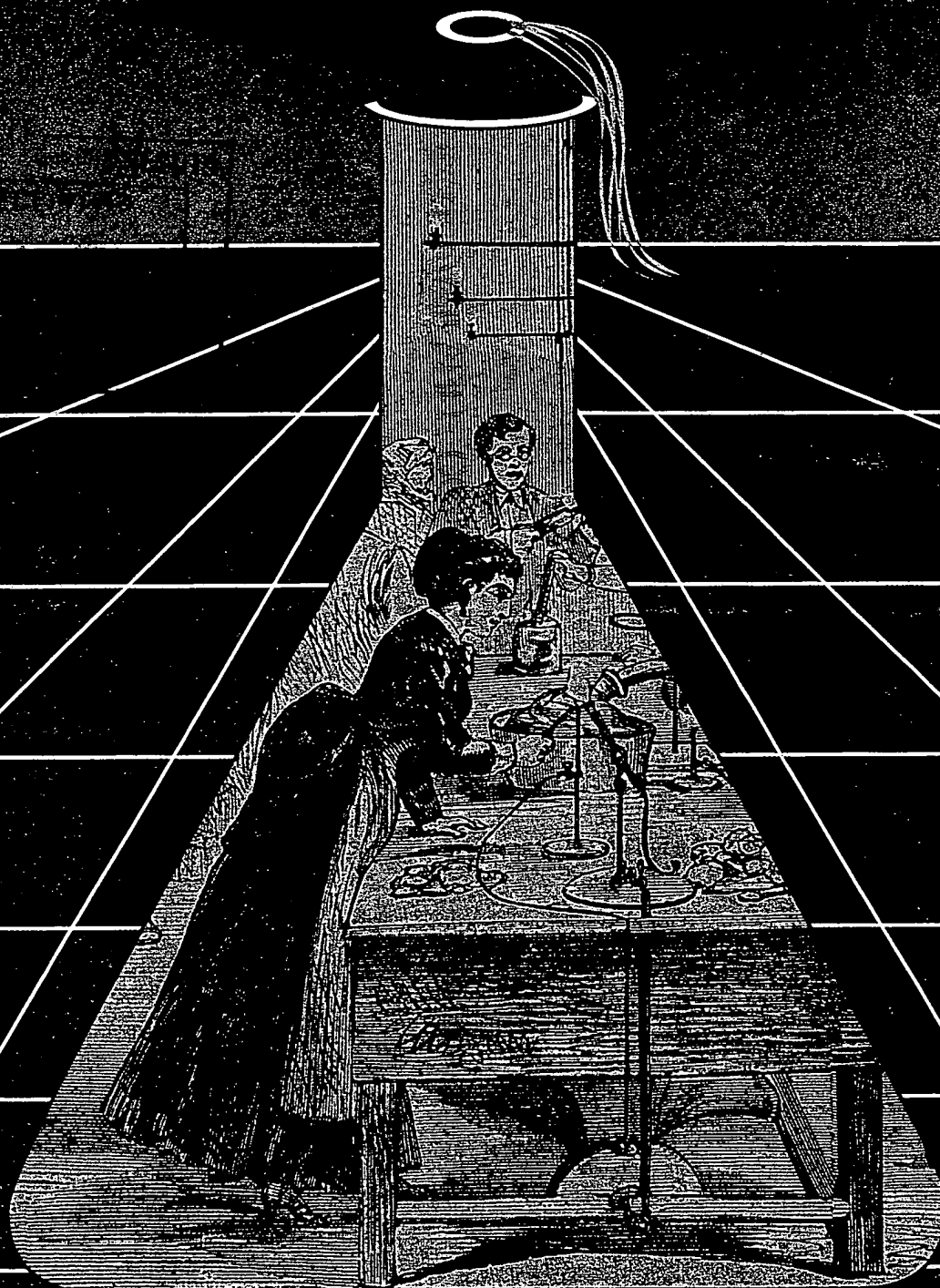


OCTOBER 15, 1991

# Analytical CHEMISTRY



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Past, Present, and Future

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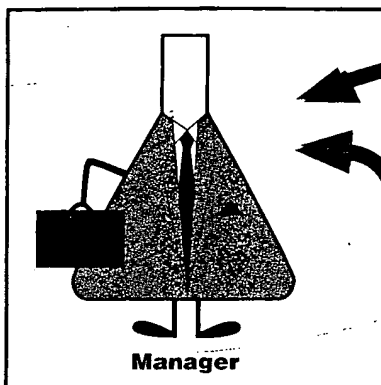
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**Classical analysis.** Since the 1940s classical analysis has taken a back seat to instrumental analysis. Charles M. Beck II of the National Institute of Standards and Technology traces the history of chemistry and argues that a revival of teaching classical analysis is vital to the interests of American industry

**On the cover.** What analytical chemistry students are taught ultimately defines the discipline. With this issue, ANALYTICAL CHEMISTRY begins an examination of the educational issues and topics that will shape the future of measurement science. (Engraving of chemistry laboratory, MIT, c. 1880, courtesy Smithsonian Institution.)

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# Supercritical Fluid Extraction and Chromatography for the Determination of Oligomers in Poly(ethylene terephthalate) Films

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Experimental dynamic supercritical fluid extraction and chromatography of lower oligomers from poly(ethylene terephthalate) films has been carried out both off-line and on-line. Quantitative results for kinetics of extraction of the cyclic ethylene terephthalate trimer have been obtained. Models for continuous (off-line) and discontinuous (on-line) extraction from films are developed and also a model for continuous extraction with nonuniform initial concentration distribution of extractable material across the film. The off-line experimental results compare satisfactorily with the models and indicate that only part of the cyclic trimer is extractable by a supercritical fluid and that the extractable material is distributed toward the surface. Discussion is made on the comparison with Soxhlet extraction and also as to why the on-line experimental results are not suitable for quantitative analysis.

Supercritical fluid extraction (SFE) is becoming an important tool in analytical science and has seen rapid development in the last few years (1). Manufacturers are now producing instrumentation designed for routine application and it often has advantages over liquid extraction.

This paper describes the application of SFE to polymer films by both off-line and on-line procedures. In off-line SFE, the sample is subjected to a flow of fluid, usually at constant temperature and pressure, and a series of samples collected at regular time intervals from the eluting fluid after depressurizing, by passing it through a solvent, for example. These samples are analyzed later. In typical on-line SFE, the SFE instrument is coupled directly to the analytical instrument, as in the SFE-SFC (supercritical fluid chromatography) method described below. Here the sample is extracted by a flowing stream of fluid at a particular temperature and pressure for a certain length of time and the extract deposited, after depressurizing, on the front of an SFC column. The extraction is then stopped, while chromatographic analysis is carried out. The two processes are then repeated alternately. From the viewpoint of modeling the extraction, the appropriate classification is into continuous and discontinuous SFE. In the SFE studies described below, off-line is continuous and on-line discontinuous, but this need not necessarily be the case. For example, in on-line SFE-SFC, it is possible to arrange continuous extraction using two columns.

**Extraction and Analysis of Poly(ethylene terephthalate) Films.** It has been shown by numerous workers that poly(ethylene terephthalate) (PET) contains small amounts of residual reactants and oligomers of low molecular weight, which are byproducts formed during the condensation polymerization of ethylene glycol (E) and terephthalic acid

(T), where the letters in enclosures indicate monomer residues. There are five main series; two cyclic series with the general formulas  $(ET)_n$  and  $(EET)-(ET)_n$ , i.e. a ring containing two adjacent ethylene glycol residues, and three linear series with the general formulas  $(ET)_n$ ,  $E(ET)_n$ , and  $T(ET)_n$ . The cyclic trimer  $(ET)_3$  has been found to make up the majority of these oligomers and was first separated and identified from PET film by Ross et al. in 1954 (2). Since then a wide variety of chromatographic methods have been used to identify and analyze the oligomers, including paper chromatography (3), liquid column chromatography (4), thin-layer chromatography (5-9), gel-permeation chromatography (10-14), gas chromatography (GC) for the very low oligomers (15) or after derivatization (16, 17), and HPLC (18-22), which is now the preferred method.

The extraction method generally used prior to analysis has been a Soxhlet extraction (up to 24 h) using chloroform, 1,4-dioxanes, toluene, or xylenes. Cooper and Semlyen (12) precipitated the high polymers from a solution of PET in 1-methylnaphthalene, leaving the lower oligomers behind in solution. Other similar precipitation methods are described with trifluoroacetic acid (21) and a mixture of 1,1,1,3,3,3-hexafluoro-2-propanol and methylene chloride (22).

Both SFE and SFC have been used in the analysis of additives and lower oligomers in polymers. SFC has become an important technique in the analysis of additives (23) and in the separation and distribution of oligomers (24). For the latter application, it extends the range of molar mass above that available by GC and has advantages in separation efficiency and detection compared with HPLC. The range of polymeric material analyzed successfully is wide, including polystyrene (25-28), polymethylsiloxanes (29), 2-vinylnaphthalene oligomers (30), and polyethylene glycols (24). Successful applications of SFE to polymers are now also reported. Hirata et al. (31) used SFE with carbon dioxide followed by HPLC and obtained complete extraction of additives in polyethylene films in under 2 h, compared with 24 h for a Soxhlet extraction. For extraction of 2,6-di-*tert*-butyl-4-methylphenol (BHT) from polypropylene pellets 3 mm in diameter, a good estimate of the concentration was obtained in 8 h, using the extrapolation methods described below, although a 99% extraction would take an estimated 80 h (32). SFE can be easily coupled to both SFC (33, 34) and GC (35, 36).

**Modeling of Extraction.** For extraction to be successful, the solute must, firstly, be sufficiently soluble in the supercritical fluid and, secondly, be transported sufficiently rapidly, by diffusion or otherwise, from the interior of the matrix in which it is contained. Of these two factors, the latter is of the most concern in analytical extractions, because in most applications the analyte is present in small quantities in the

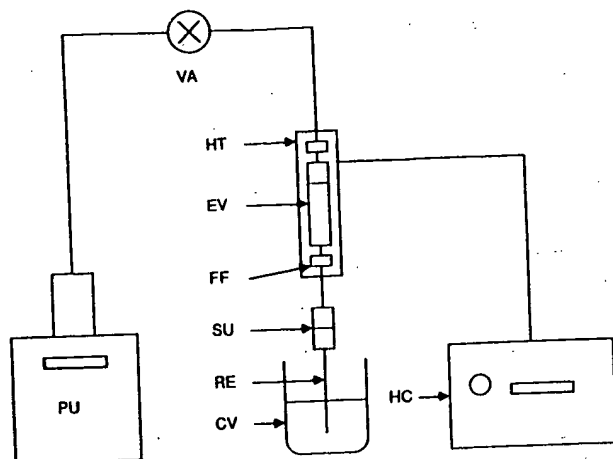


Figure 1. Off-line SFE system: PU, CO<sub>2</sub> pump; VA, on/off valve; HT, thermostated tube; FF, fingertight connectors; EV, extraction vessel; SU, Swagelock union; RE, restrictor; CV, collection vial; HC, heater controller.

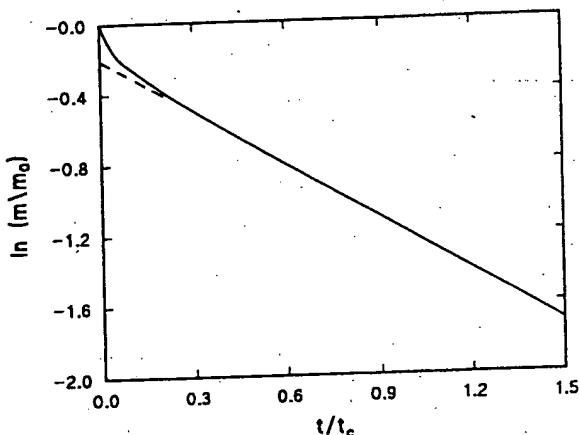


Figure 2. Theoretical curve of  $\ln(m/m_0)$  versus time for continuous dynamic SFE of a film with uniform initial concentration distribution.

matrix and a fluid and conditions have been chosen in which the analyte is soluble. The models in this paper therefore assume that the concentration of solute is essentially zero. The effect of solubility limitation on the extraction process has been discussed elsewhere (37).

In a recent study of continuous SFE of powdered matrices (32), experimental measurements were compared with a simple model, described as the hot-ball model. This model assumes that the matrix particles are spheres, that the initial distribution of the solute within the spheres is uniform, and that the flow rate is high enough for the concentration of the solute in the supercritical fluid to be considered effectively zero. The kinetics are described in terms of  $m$ , the amount of solute present in the matrix at a given time compared with  $m_0$ , the initial amount, and the model predicts the form of the curve of  $\ln(m/m_0)$  versus time. In spite of the simplicity of the model, a variety of real systems were found to exhibit most of the characteristics of the theoretical curve. These are that the curve falls steeply initially but then becomes linear and that, if the slope of the linear portion is  $-1/t_c$ , the curve becomes linear after a time of approximately  $0.5t_c$ . The intercepts of the linear portions of the curves for real systems, however, were different from the theoretical value of  $-0.5$ . These differences were explained qualitatively in terms of (a) the irregular shape of the matrix particles, which lowers the intercept, (b) limitation by solubility, which raises the intercept, and (c) nonuniform extractable solute distribution, which lowers the intercept if it is concentrated near the surface and vice versa.

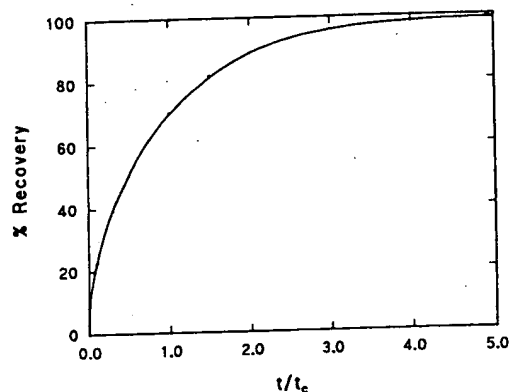


Figure 3. Theoretical curve of percentage extracted versus time for continuous dynamic SFE of a film with uniform initial concentration distribution.

The exponential behavior of the extraction after the initial period, allows the use of extrapolation to obtain quantitative analytical information in a shorter time than for exhaustive extraction. If extraction is carried out at least as long as the initial nonexponential period to obtain an extracted mass  $m_1$ , followed by extraction over two subsequent equal time periods to obtain masses  $m_2$  and  $m_3$ , then it can be readily shown that  $m_0$ , the total mass in the sample, is given by

$$m_0 = m_1 + m_2^2 / (m_2 - m_3) \quad (1)$$

This equation has been used, for example, in the analysis by SFE of polymer pellets (32) and, as will be shown below is applicable to SFE of films in some cases.

## EXPERIMENTAL SECTION

**Off-Line SFE/Open-Tubular SFC.** The system used is shown in Figure 1. A Varian 8500 or a Lee Scientific 602D syringe pump, with cooled heads was used to provide pure CO<sub>2</sub> (BOC) under pressure, PU. This was fed to an extraction vessel, EV, of 6.94-mL internal volume, fitted with fingertight, slip-free connectors for easy installment (Keystone Scientific), FF. Stainless-steel frits, 3- $\mu$ m pore diameter, were placed at either end of the cell and stainless steel tubing was used to connect the cell, via an on-off valve, VA, to the pump. The other end of the cell was connected to a piece of deactivated silica restrictor, RE, 100 mm  $\times$  20  $\mu$ m i.d. (Polymicro Technologies) using a graphitized vespel ferrule and a Swagelock union (1/16 in.), SU. A uniform-bore restrictor was used to minimize the possibility of solute deposition within it. Such holdup of solute appears to happen when larger amounts are being extracted and there are large and random variations in amounts obtained in adjacent time periods. No evidence of solute holdup in the restrictor was apparent in these experiments. The extraction cell was placed in a heated ceramic tube, built in-house, HT, which had its temperature controlled by a heater controller, HC.

A 2–3-g sample of PET film, 250  $\mu$ m in thickness, was cut into strips (5 mm  $\times$  100 mm), and the surface was wiped lightly with acetone on a paper tissue, dried, and placed lengthwise in the cell. The cleaning procedure was to remove lubricant used in the manufacture of the film and other surface contaminants and is a procedure used routinely by the manufacturers in a number of types of analysis. The strips were attached together at both ends by thin nickel wire, and aluminum spacers were placed between the strips. This arrangement allowed free passage of the supercritical fluid along the surfaces of the film. Extractions were performed at 70 °C and 400 atm, the pressure being measured at the pump. The effluent from the silica tubing was passed into ca. 5 mL of HPLC grade dichloromethane in a collection vial, CV, and the vial was changed every 30 min. A solution of Irganox 3114 (tris(3,5-di-*tert*-butyl-4-hydroxybenzyl) isocyanurate) in dichloromethane was added to each vial, as an internal standard, and the solutions were then evaporated down to ca. 200  $\mu$ L, by passing nitrogen gas slowly over the surface and injected into a

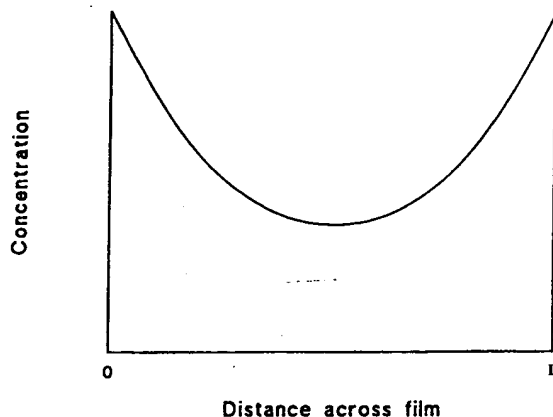


Figure 4. Model distribution of the concentration of extractable material across a film.

Lee Scientific 602D SFC instrument. A 1-s time-split injection was used, injecting ca. 100-nL onto a 10 m  $\times$  50  $\mu$ m i.d. column with a cross-linked film of 30% biphenyl-substituted methyl polysiloxane (SB-Biphenyl-30, Dionex). Restriction was achieved with a frit restrictor (Dionex), giving a flow rate of 17 mm s<sup>-1</sup> at 70 °C and 75 atm. Chromatography was performed at 130 °C using a pressure ramp from 100 to 400 atm at 10 atm/min. The flame ionization detector was maintained throughout at 400 °C.

**On-Line SFE/Open-Tubular SFC.** A Lee Scientific 602D SFC system was combined with an on-line Lee Scientific SFE unit. Pure CO<sub>2</sub> was used, for which the purity problems are discussed below. About 15 mg of PET film, 250  $\mu$ m in thickness, was cut into strips (3 mm  $\times$  10 mm), and the surface was wiped lightly with acetone on a paper tissue, dried, and placed in the extraction cell. SFE was carried out at 70 °C and 400 atm for periods of 30 min. Analytes from the extracts were collected at atmospheric pressure in a trap cooled to 45 °C by using industrial grade solid CO<sub>2</sub>. SFC was then carried out with the parameters described in the last section over a period of 30 min. The extraction-chromatography procedure was repeatedly carried out on the same sample.

For on-line SFC a calibration curve of peak area was obtained by placing known volumes of standard solutions of the cyclic trimer in the extraction cell and carrying out the extraction procedure for 5 min. Calibration covered the range required for the extraction experiments and was from 0.01 to 1.0  $\mu$ g. A straight line with no observable scatter was obtained. No direct injection calibrations were carried out. To estimate the precision of an individual on-line extraction, initial extractions on 18 samples of PET of 15.1  $\pm$  0.2 mg gave a coefficient of variation of 8%.

Low levels of impurities in the CO<sub>2</sub> used, mainly hydrocarbons, can cause significant problems in coupled SFE-SFC, because relatively large amounts (ca. 1 g) of CO<sub>2</sub> are used, from which the impurities are collected in the trap. The SFC grade CO<sub>2</sub> (Air Products) supplied as liquid via a dip-tube gave a large signal from a blank 30-min extraction. The situation was much improved when gas from the head of the cylinder was used and condensed into the pump. Houben et al. had observed a similar improvement and explained this on the basis that the impurities, which are less volatile than CO<sub>2</sub>, are more concentrated in the liquid phase than the gas in the cylinder (38). Further improvement was obtained by adding an activated charcoal trap with 30–60 mesh particles (Dionex) to the original basic alumina trap (Dionex) and also an oxygen trap (Phase-Sep) between these two traps to remove sulfides. With all these modifications, a 30-min blank extraction gave a negligible rise in the baseline of the subsequent chromatogram.

## MODELS FOR SFE OF FILMS

**Continuous Extraction with Uniform Initial Concentration.** For extraction from a thin film of well-defined geometry, an appropriate model is that of an infinite slab of thickness  $L$ . An initial uniform distribution of the compound to be extracted is assumed with a diffusion coefficient of  $D$ . As the slab is infinite, we must consider  $m$  to be the amount

Table I. Values for the Intercept,  $I$ , for Extractions from a Film with Nonuniform Initial Solute Distribution for Various Values of the Ratio of the Distance Parameter for the Distribution,  $a$ , to the Thickness of the Film,  $L$

$a/L$	$I$	$a/L$	$I$
	0.2100	0.05	1.6338
1	0.2277	0.01	3.2199
0.5	0.2779	0.005	3.9120
0.3	0.3820	0.001	5.5215
0.1	1.0103		

of the compound in a section of the slab, of given area, at time  $t$  and  $m_0$  to be the amount in the same section at  $t = 0$ . Treatment of this model involves solving the diffusion or Fourier equation for this particular geometry, with appropriate boundary conditions. Solutions for the Fourier equation for various geometries and boundary conditions are given by Carslaw and Jaeger (39), in the context of heat conduction (where the same equation applies) and also by Crank (40), who has translated Carslaw's equations into diffusion notation. Adaptation of the appropriate solutions gives

$$m/m_0 =$$

$$(8/\pi^2) \sum_{n=0}^{\infty} (1/(2n+1)^2) \exp(-(2n+1)^2 \pi^2 D t / L^2) \quad (2)$$

where  $n$  is an integer. If a characteristic time for the extraction,  $t_c$ , is defined by

$$t_c = L^2 / \pi^2 D \quad (3)$$

eq 2 may be simplified to

$$m/m_0 = (8/\pi^2) [\exp(-t/t_c) + (1/9) \exp(-9t/t_c) + (1/25) \exp(-25t/t_c) + \dots] \quad (4)$$

This is a sum of exponential decays, similar to that obtained for the spherical model, with the first exponential term in the square brackets becoming dominant at longer times. This equation gives, after factorization of  $\exp(t/t_c)$  from the square brackets, taking natural logarithms, and substituting the numerical value of  $\ln(8/\pi^2)$

$$\ln(m/m_0) = -0.2100 - t/t_c + \ln[1 + (1/9) \exp(-8t/t_c) + (1/25) \exp(-24t/t_c) + \dots] \quad (5)$$

A plot of  $\ln(m/m_0)$  versus time, shown in Figure 2, therefore becomes linear at longer times and the nature of the equations is such that this linear portion is reached more rapidly than in the case of a sphere. This is a feature of the lower surface to volume ratio for the infinite slab (not including the edges), as compared with the sphere. The approximate equation for this model at longer times is

$$\ln(m/m_0) = -0.2100 - t/t_c \quad (6)$$

The straight line portion of Figure 2, which is continued to the  $t = 0$  axis as a dashed line, is a plot of eq 6, with an intercept of  $-0.2100$ . The linear behavior at longer times means that the extrapolation method based on eq 1, developed for the hot-ball model, is also applicable to continuous extraction from films.

Qualitatively, the theoretical curve of percentage extracted versus time for an infinite slab is similar to that for a sphere and exhibits the same long tail, as is shown in Figure 3. The material (37%) is extracted during an initial period of  $0.25t_c$ . The time required to extract 99% of the material, however, is  $4.4t_c$ , i.e. 17 times the time needed to extract the first 37%.

**Continuous Extraction with Nonuniform Initial Concentration.** In the case of extraction from a film of

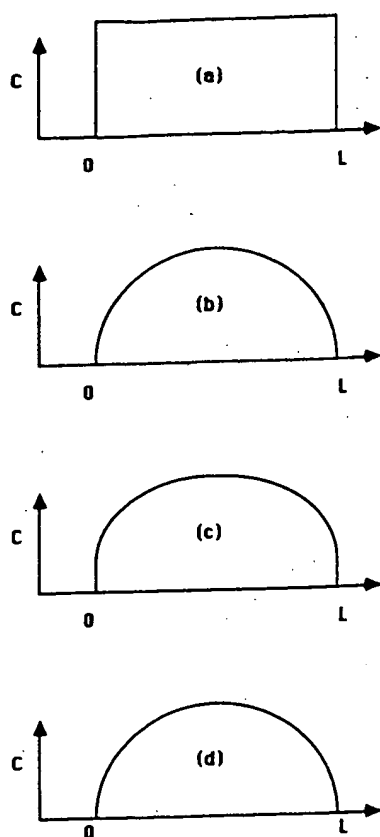


Figure 5. Development of concentration profiles during discontinuous (on-line) extraction (schematic).

known geometry, it is worthwhile to investigate the effect of nonuniform distribution on the theoretical results. A simple model for such a distribution is an exponential fall-off in concentration from each surface. The concentration then is a function of the distance  $x$  along a coordinate perpendicular to the slab surface. If the slab surfaces are located at  $x = 0$  and  $x = L$ , then the concentration is initially given by

$$c(x,0) = c_0[\exp\{-x/a\} + \exp\{-(L-x)/a\}] \quad (7)$$

where  $a$  is a distance parameter, determining the rate of fall off from the surface. This profile is shown in Figure 4 for a value of  $a/L$  of 0.3. The average concentration in the slab, obtained by integrating eq 7, with respect to  $x$  between 0 and  $L$ , and dividing by  $L$  is

$$c_{av}(0) = (2c_0/L)[1 - \exp(-L/a)] \quad (8)$$

Adaption of the appropriate heat conduction equation (39) gives an integral equation for determining the concentration profile at a later time,  $c(x,t)$ , from an initial concentration profile  $c(x,0)$ , when the surfaces of the infinite slab are maintained at zero concentration by continuous extraction unlimited by solubility. Substitution of  $c(x,0)$  from eq 7 and integration leads to an expression for  $c(x,t)$ , which is then further integrated with respect to  $x$  and divided by  $L$  to give the average concentration across the slab at  $t$ , as follows:

$$c_{av}(t) = 8c_0[1 + \exp(-L/a)] \sum_{n=0}^{\infty} [1/(L/a)^2 + \pi^2(2n+1)^2] \exp\{-(2n+1)^2 t/t_c\} \quad (9)$$

Since

$$m/m_0 = c_{av}(t)/c_{av}(0) \quad (10)$$

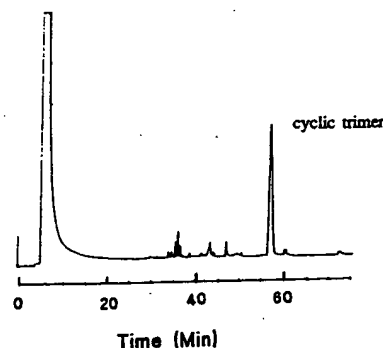


Figure 6. Open-tube SFC chromatogram of PET extract from off-line extraction.

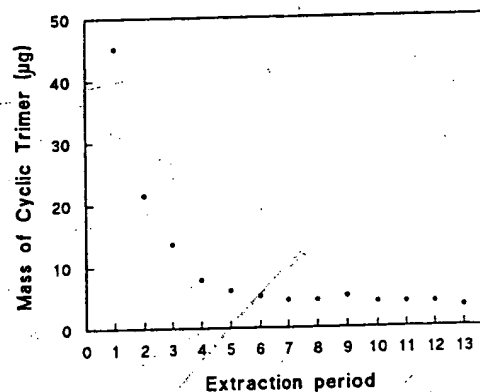


Figure 7. Mass extracted during each 30-min period of off-line, continuous SFE of cyclic trimer from PET.

$$m/m_0 = (4L/a)[\{1 + \exp(-L/a)\}/\{1 - \exp(-L/a)\}] \times \sum_{n=1}^{\infty} [1/(L/a)^2 + \pi^2(2n+1)^2] \exp\{-(2n+1)^2 t/t_c\} \quad (11)$$

This equation is of the same type as eq 4, derived for uniform initial concentration distribution, with a sum of exponential decays of which the first term becomes dominant at longer times, i.e.  $\ln(m/m_0)$  becomes linear with time at longer times. Also it can be readily shown that in the limit of very large values of  $a/L$ , which correspond to uniform distribution of concentration initially, eq 11 becomes identical with eq 4, as required. The long-time behavior means that the extrapolation formula, eq 1, may also be used in this case.

However, for finite values of  $a/L$ , and especially for values less than unity, the higher terms in the sum of exponentials fall off less rapidly in importance than was the case for uniform initial distribution. As a result, plots of  $\ln(m/m_0)$  are similar in form to that of Figure 2, except that the initial rapid extraction is of greater importance and there is a lowering of the intercept of the linear portion. The value of the intercept,  $-I$ , may be found from the natural logarithm of the coefficient of the first term in the series ( $n = 0$ ), i.e.

$$-I = \ln \{ (4a/L)[\{1 + \exp(-L/a)\}/\{1 - \exp(-L/a)\}] \times [1/(1 + (\pi a/L)^2)] \} \quad (12)$$

Some values of  $I$  for various values of  $a/L$  are given in Table I. For a value of  $a/L$  of 0.3, which is the profile shown in Figure 4, the intercept is  $-0.39$ .

**Discontinuous SFE with Uniform Initial Concentration.** It is a much more difficult problem to treat on-line analyses, where typically extraction is carried out for a certain period, following which extraction is stopped and analysis, e.g. chromatography, is carried out for a further period, these two processes then being repeated alternately several times.

**Table II. Extrapolation To Obtain Final Quantities in the Off-Line Extraction of Cyclic Trimer from PET**

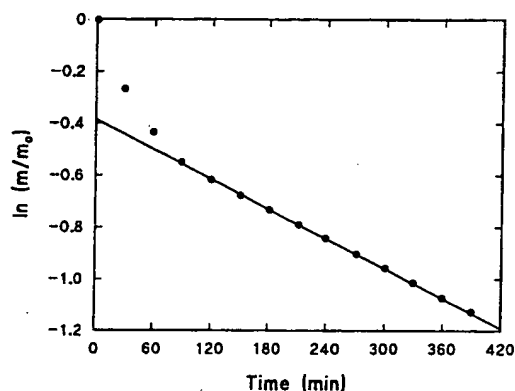
extraction times, min	weight extracted, $\mu\text{g}$	results predicted from eq 1, $\mu\text{g}$
0-150	93.88	
150-270	19.08	
270-390	15.44	193.88
0-210	103.47	
210-300	13.61	
300-390	11.32	184.36
0-270	112.96	
270-330	8.14	
330-390	7.30	191.91

Qualitatively, this type of experiment is illustrated in Figure 5, which shows schematically the development of the concentration profile of an extracted material across the film. The first period would be identical with off-line analysis, with initial uniform concentration, shown in (a), developing to the profile in (b). During the analysis period, when extraction ceases, diffusion of material will continue to occur within the film down the concentration gradient, i.e. toward the surface, producing the profile shown in (c). After extraction the profile will develop to (d), and so on. The extent of diffusion toward the surface during the analysis will depend on the length of the analysis (nonextraction) period. This will subsequently effect the amount extracted in a given time during the next extraction, as is shown by experiment.

In this section equations for discontinuous extraction are developed for a particular case: the infinite slab with a uniform initial concentration distribution and with no solubility limitation. We consider a series of  $N$  extractions, for periods of time  $e_1, e_2, \dots, e_N$ , alternated with  $N-1$  periods,  $r_1, r_2, \dots, r_{N-1}$ , in which no extraction takes place. It will be necessary to calculate the development of the concentration profile along the direction perpendicular to the slab surfaces,  $c(x,t)$ , during these periods. During extraction, this may be done with the aid of an equation used in the last section. For the periods where there is no extraction an appropriate equation can also be adapted from Carslaw and Jaeger (39), which describes the development of  $c(x,t)$  under conditions where there is no flux of compound through the slab surfaces. Starting with uniform concentration,  $c_0$ , which is also equal to the average concentration,  $c_{av,0}$ , the equations for the two sets of boundary conditions are alternately applied for the appropriate periods of time. Also after each extraction period, the concentration is averaged over the slab by integration to give a value of  $c_{av,N}$ . This quantity is then divided by  $c_{av,0}$  to give, by virtue of eq 10, a value of  $m/m_0$  after the  $N$ th extraction,  $(m/m_0)_N$ . The integrations necessary for this procedure are straight-forward, but regrettably each application of the equations results in an additional summation being introduced into the formulations. The results can be represented by the following general formula:

$$(m/m_0)_N = (8/\pi^2)N \sum_{i_1=0}^{\infty} \dots \sum_{i_N=0}^{\infty} \sum_{j_1=1}^{\infty} \dots \sum_{j_{N-1}=1}^{\infty} \prod_{k=1}^N \left[ \frac{1}{(2i_k+1)^2} \exp\left\{-(2i_k)e_k/t_c\right\} \frac{1}{(2j_{k+1}+1)^2} + \frac{(2i_k+1)^2}{(2i_k+1)^2 - (2j_k)^2} \frac{2 \exp\{-(2j_k)^2 r_k/t_c\}}{(2j_{k+1}+1)^2 - (2j_k)^2} \right] \quad (13)$$

This formula is obviously unsuitable for routine analytical application, but it may be simplified if it is arranged such that

**Figure 8. Off-line SFE of cyclic trimer from PET.**

the experimental periods,  $e_i$  and  $r_i$ , are long enough, compared with  $t_c$ , so that only the first term in each sum is important. Comparison with the situation for continuous extraction, indicates that the periods should be at least  $0.25t_c$  for this approximation to be valid. In this case eq 13 becomes

$$(m/m_0)_N = (8/\pi^2)^N \left\{ \prod_{k=1}^N \exp(-e_k/t_c) \right\} \left\{ \prod_{i=1}^{N-1} [1 + (2/9) \exp(-r_i/t_c)] \right\} \quad (14)$$

If all the extraction periods are a fixed value,  $t_e$ , and all the periods between extraction are also a fixed time,  $t_r$ , the equation simplifies further to

$$(m/m_0)_N = \left\{ (8/\pi^2)^N \exp(-t_e/t_c) \right\}^N \{1 + (2/9) \exp(-t_r/t_c)\}^{N-1} \quad (15)$$

Thus, after the first extraction, the value of  $m/m_0$  falls exponentially with the number of extractions, and this can be seen by taking the natural logarithm of eq 15 to give

$$\ln \{(m/m_0)_N\} = -N \ln \{1 + (2/9) \exp(-t_r/t_c)\} + \{N \ln (8/\pi^2) - t_e/t_c + \ln \{1 + (2/9) \exp(-t_e/t_c)\}\} N \quad (17)$$

A plot of  $\ln \{(m/m_0)_N\}$  against the number of extractions,  $N$ , should therefore give a straight line with an intercept of  $-\ln \{1 + (2/9) \exp(-t_r/t_c)\}$ . Experience with continuous extraction indicates that the intercept may be different from this value for various reasons.

Equation 17 may be suitable for the analysis of discontinuous extraction data in some cases and indicates that the extrapolation procedure of eq 1 may be applicable also in these cases. However, in the present study the lengths of time needed for the extraction and nonextraction periods are impractically long at ca. 125 min. To obtain quantitative analytical results using discontinuous on-line SFE thus may pose problems.

## RESULTS AND DISCUSSION

**Off-Line SFE/SFC of PET Film.** Off-line extraction of a PET sample film of 2.739-g weight and 250- $\mu\text{m}$  thickness was carried out by the procedures described above for a series of 13 consecutive 30-min extractions, i.e. over a period of 6.5 h. The extracts were analyzed by SFC, as described above, and a typical chromatogram is shown in Figure 6, illustrating the dominance of the cyclic trimer in the extract. Raw data obtained from the analyses are plotted in Figure 7, which gives the weight of cyclic trimer obtained in each of the successive extraction periods. As can be seen, the extraction is by no means exhaustive, and the extraction is exhibiting the typical long tail.



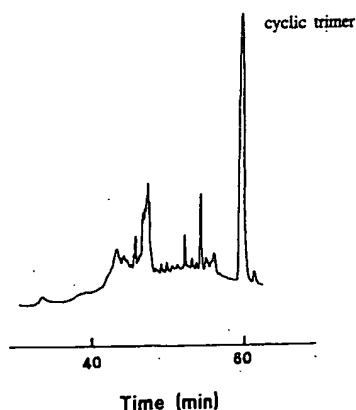


Figure 9. Open-tube SFC chromatogram of the PET extract from on-line extraction.

As the initial rapid extraction is apparently over after about 150 min, the extrapolation procedure of eq 1 is applied such that the period for  $m_1$  is not less than 150 min. Three combinations of times are shown in Table II, satisfying this condition and where the two subsequent periods are equal. The raw data, summed for these periods are also shown, as are the results obtained by applying eq 1. The results are in reasonable agreement with an average of  $190 \pm 5 \mu\text{g}$  or 3%. This is in spite of some scatter in the raw data, which is smoothed to some extent by the summation used in the procedure. The final result compares with  $128 \mu\text{g}$  obtained experimentally over the whole extraction period. It can be calculated that about 27 h would be required to extract 97% of the material and thus obtain the same accuracy without using the extrapolation method.

Using the extrapolated value for  $m_0$ , values of  $\ln(m/m_0)$  were calculated and plotted against time in Figure 8. The curve has the same form as Figure 2 and, with the slope of the linear portion taken to be  $-1/t_c$ ,  $t_c$  was calculated to be 506 s. This gives  $0.25t_c$  as 127 s, and this is seen to be the approximate time at which the curve becomes linear, as predicted by the model. However, the experimental intercept, at  $-0.39$ , is different from the theoretical value of  $-0.21$ . This can be explained on the basis that the extractable material is nonuniformly distributed and is more concentrated toward the surface of the film. Calculations carried out earlier for a nonuniform distribution of exponential form, show that the distribution illustrated in Figure 4 would give an intercept of  $-0.38$ , close to this value, and so, while not necessarily giving the exact shape of the distribution for the system studies, Figure 4 does indicate the extent of the nonuniform distribution.

The total amount of cyclic trimer extracted from PET at  $6.9 \times 10^{-3}$  wt % is considerably less than that obtained by Soxhlet extraction, which is in turn less than that obtained by dissolution followed by precipitation of the higher oligomers, all on similar samples. The extraction appears to be tailing off as predicted by the model, however, and reaching the amount predicted by eq 1. Apparently, only a small proportion of the cyclic oligomer is extractable by SFE. This is in contrast to the SFE of the much smaller molecule BHT from standard polypropylene pellets, which appears to be quantitative (32). It seems likely that many of the larger cyclic trimer molecules are locked into the polymer matrix and not accessible to SFE. This may also be a feature of the high degree of crystallinity exhibited by PET and the fact that SFE was carried out not significantly above the glass transition temperature of the sample, which was  $69^\circ\text{C}$ .

The greater efficiency of Soxhlet extraction may be due to the fact that the extraction is carried out above the glass transition temperature and that the polymer is swollen by the

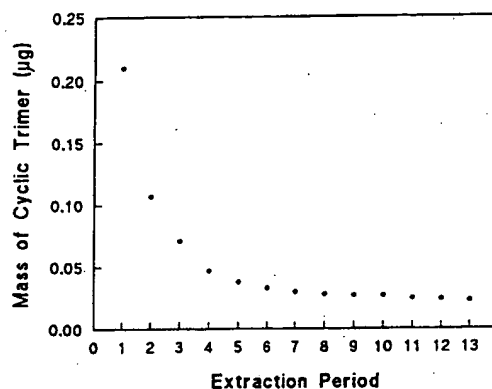


Figure 10. Mass extracted during each 30-min period of on-line, discontinuous SFE of cyclic trimer from PET.

solvent, releasing more of the trimer. The theory of liquid extraction is parallel to that for SFE, and so there is no basis in the models for the difference. Although no detailed study of the kinetics of Soxhlet extraction appears to have been carried out, extraction by liquids in industrial processes is based on similar considerations and equations to those above (41, 42).

In any fluid extraction of polymers, the eventual amount extracted may be quantitative for small molecules, essentially zero for higher oligomers, while for molecules of intermediate size a fraction will be obtained. This fraction will depend on the type of extraction and the conditions used. SFE can only be used to obtain quantitatively the amounts of additives or lower oligomers in a polymer in some cases. Nonquantitative amounts extracted by SFE may, however, be relevant for some considerations, such as the migration of substances from polymer containers into foodstuffs.

**On-Line SFE/SFC of PET Film.** On-line extraction of a similar PET sample film of ca. 15 mg was carried out by the repetitive procedure described above for a series of 13 consecutive 30-min extractions, each followed by a 30-min period for SFC analysis, during which extraction was stopped. A typical chromatogram obtained is shown in Figure 9, which has no solvent peak and poorer resolution because of the larger effective injection volume inherent in the trapping system. The raw data obtained from the analyses are plotted in Figure 10, which gives the weight of cyclic trimer obtained in each of the successive extraction-chromatography periods. Although superficially similar to the data in Figure 7, consistent results using the extrapolation procedure could not be obtained, indicating that the data would not follow the curve of Figure 2. From the discussions of modeling discontinuous extraction above, this would be expected, as the extraction and nonextraction periods are 30 min, whereas  $0.25t_c$  is greater at 125 min. Thus further analysis of the present data cannot be achieved easily and to collect data with extraction and analysis times of greater than 125 min, as is necessary for this thickness of film, would make the procedure impracticably long.

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## CORRESPONDENCE

### Separation of cis and trans Double-Bond Isomers Using Capillary Zone Electrophoresis

Sir: Capillary electrophoresis continues to emerge as a powerful separation technique (1). Examples of the use of capillary electrophoresis for the separation of nearly identical pairs of molecules have appeared in the literature, including the separation of isotopomers (2), *d* and *l* optical isomers (3-5), and positional substitution isomers (6). Another class of isomeric compounds that may be amenable to separation via capillary electrophoresis is that of the cis and trans double-bond isomers.

Double-bond isomer separation is an important analytical problem in the area of retinoid chemistry. Retinoids are involved in many major biological functions such as growth promotion, reproduction, and differentiation and maintenance of epithelial tissue (7). Retinoids also serve as visual pigments that play a critical role in photoreception and phototransduction (8). They also play a pivotal photosynthetic function for the transmembrane protein, bacteriorhodopsin, in halophilic bacteria (9).

Therapeutically, retinoids have been used to treat a number of clinical skin conditions, including cystic acne and various forms of ichthyosis, Darier disease, and psoriasis (10-12). The effect of retinoids in the prevention of cancer has also been studied (13, 14). Since cis and trans isomers of a given retinoid may not have the same biological activities (15-17) and because these compounds can undergo photochemically induced double-bond isomerization (18), an analytical technique ca-

pable of separating such isomers is necessary. Additionally, it is desirable for this technique to be sensitive enough to analyze small quantities of material, especially for synthetically derived retinoid analogues. Capillary electrophoresis, a technique that can analyze picogram amounts of material, has the potential of meeting these analytical needs.

Because of their configurational difference, one might expect that cis and trans double-bond isomers would possess different hydrodynamic radii. While the charge carried by such isomers is the same (at a suitable pH), the electrophoretic mobilities would be different due to the differing hydrodynamic radii, thus allowing for an electrophoretic separation. We report here the baseline-resolved separation of the model double-bond isomers fumaric acid (*trans*-butenedioic acid) and maleic acid (*cis*-butenedioic acid) and the separation of the prototypical retinoid isomers all-*trans*-retinoic acid and 13-*cis*-retinoic acid (Figure 1), in free solution using capillary zone electrophoresis.

#### EXPERIMENTAL SECTION

**Materials.** Fused silica, polyimide-coated capillary tubing having dimensions of 375  $\mu\text{m}$  o.d. and 75  $\mu\text{m}$  i.d. was purchased from Polymicro Technologies (Phoenix, AZ). ACS reagent-grade sodium tetraborate decahydrate and boric acid were obtained from Mallinckrodt (Paris, KY). Fumaric acid (99+%) and maleic acid (99%) were obtained from Aldrich Chemical Co. (Milwaukee, WI).

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